

NASA CR-1660, 3^e

NASA CONTRACTOR REPORT 166357

NASA-CR-166357
19820020112

Plant Diversity to Support Humans
In a CELSS Ground-Based Demonstrator

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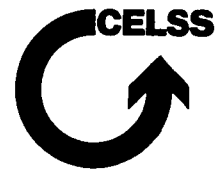
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NASA Grant NSG-2401
June 1982

NASA



NF02619



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Prepared for
Ames Research Center
Under NASA Grant NSG-2401



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Space Administration

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N82-27988

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Introduction

The National Aeronautics and Space Administration (NASA) has considered a controlled ecological life support system (CELSS) for human habitation in preparation for future long duration space flights. The success of such a system will likely depend upon the feasibility of revitalization of food resources and the human nutritional needs which are to be met by these food resources. Edible higher plants would be prime candidates for the photoautotrophic components of this system if nutritionally adequate diets can be derived from these plant sources to support humans (43).

Our tasks are to develop a human nutritional requirements information based on current knowledge, for inhabitants envisioned in the CELSS ground-based demonstrator; and to identify groups of plant products that can provide the nutrients.

Certain aspects of these tasks have been reported in "Nutritional and Cultural Aspects of Plant Species Selection for a Regenerative Life Support System" (13). In this report we present a brief discussion regarding factors that influence the human nutritional requirements envisioned in CELSS-GD and on three bioavailability experiments of Ca, Fe and Zn. A fourth experiment on the interrelationship of protein and magnesium on Ca retention is also described.

Human Nutritional Requirements in CELSS-GD.

The best known nutritional standard is the Recommended Dietary Allowances (RDA), published by the Food and Nutrition Board of National Research Council (NRC), National Academy of Sciences, and updated at approximately five year intervals (30). The RDA are designed to serve as a guide for planning nutrient intake of population groups but may not meet requirements for particular individuals. With the exception of the allowances for energy the RDA generally exceed the requirements of the majority of persons.

In the most recent edition of RDA (30), ranges of estimated safe and adequate intakes of three vitamins (vitamin K, pantothenic acid, and biotin) six trace elements (copper, manganese, flouride, chromium, selenium, and molybdenum), and three electrolytes (sodium, potassium, and chloride) are tabulated for the first time. These ranges serve as guidelines for adequacy and also as warnings against excessive intakes through use of supplements. Scientific information on which to base these suggested intake is considered less complete than that used to determine the RDA.

Nutrient Composition of Total Plant Diets.

Most foods contain more than one nutrient individually, but no single food item contains all the essential nutrients in the amounts that are needed for optimal health. A nutritionally adequate diet could be obtained by various combinations of foods, and as the number and kinds of foods become more restrictive, it becomes increasingly difficult to formulate nutritionally adequate diets.

A total plant diet can be made nutritionally adequate by careful planning, giving proper attention to specific nutrients which may be in a less available form or in lower concentration or absent in plant foods (6). Most nutritionists have agreed that well-planned vegetarian diets are consistent with good nutritional status (1).

In order to establish a frame of reference for total vegetarian diet, we have assembled a 14-day vegetarian cycle menu (Appendix A) through communication with practicing vegetarians and other sources (19, 36).

This menu represents food items derived from fifty-six plant species (Table 1). Presently available data on food composition (47) were used to calculate one serving per menu item, three meals per day. The average nutrient contents of this cycle menu are listed in Table 2.

The estimate of nutrient intake on food composition data is not a precise procedure. While the calorie and protein contents of many individual food items may be within 10% of the calculated value, this is not true for other nutrients. In addition, factors affecting bioavailability of nutrients are generally not considered in food tables (30). Nevertheless, this estimation is helpful to provide a perspective for assessing the particular issues in foods solely of plant origin.

a. Energy. The average energy value provided from the menus is considerably lower than the level recommended by NRC for sedentary activities. Low energy intake is a general concern with vegetarians whose diets are not well planned. For inhabitants in CELSS-GD, energy intake may be increased by using more leavened breads, cereals, legumes, nuts and seeds. Between meal snacks and larger serving sizes will also correct this discrepancy.

Table 1. Selected Plant Used By Practicing Vegetarians

<u>Legume</u>	<u>Cereal</u>	<u>Fruit</u>	<u>Vegetable</u>	<u>Miscellaneous</u>
Peanuts	Rye	Orange	Potato	Mustard spice
Chestnuts	Wheat	Grapefruit	Collards	Nutmeg
Walnuts	Corn	Lemon	Rhubarb	Celery seed
Almonds	Oats	Apple	Carrots	Sesame Seed
Soybeans	Rice	Strawberry	Cabbage	Oregano
Split Peas		Peach	Asparagus	Sunflower seeds
Beans -		Pear	Green lettuce	Mint
Navy		Watermelon	Spinach	(Active Dry Yeast)
White		Grapes	Celery	Green onion
Mung			Green pepper	Garlic
Lima			Green peas	(Baking powder)
Kidney			Tomato	Parsley
Chickpeas			Green beans	Onions
Lentils			Winter squash	(Salt)
Alfalfa			Beet greens	
			Cucumbers	
			Broccoli	

Table 2. Dietary Recommendations vs. Practices

Nutrient	NRC ¹ (RDA)	FAO/WHO ²	Skylab ³	Vegetarian ⁴
Energy kcal	2700(2300-3100)	3000	RDA	1970
Protein g	56	37-62	90-125 ⁺ 10	65.4
Vit. A mcg R.E.	1000	750	RDA	2102
Vit. D mcg	5 (200 I.U.)	100 I.U.	RDA	-
Vit. E mg Alpha T.E.	10	-	RDA	-
Vit. C mg	60	30	RDA	180
Thiamin mg	1.4	1.2	RDA	1.9
Riboflavin mg	1.6	1.7	RDA	1.2
Niacin mg	18	19.8	RDA	18
Vit. B ₆ mg	2.2	-	RDA	-
Folacin mcg	400	200	RDA	-
Vit. B ₁₂ mcg	3	2	RDA	0
Calcium mg	800	400-500	750-850 ⁺ 16	594
Phosphorus mg	800	-	1500-1700 ⁺ 120	1368
Magnesium mg	350	-	300-400 ⁺ 100	-
Iron mg	10 (Man) 18 (Woman)	9 (Man) 28 (Woman)	RDA -	19
Zinc mg	15	-	RDA	-
Iodine mcg	150	-	RDA	-
Sodium g	1.1-3.3		3.0-6.0 ⁺ 0.5	2.2
Potassium mg	1525-4575		2740 Min. No Max. and No Range	4100

¹NRC: Reference Man 70 kg mixed diet

²FAO/WHO: Reference Man 65 kg mixed diet

³Skylab: Mixed diet

⁴Veg.: Average calculated values of a 14-day vegetarian cycle menu which was developed from communication with practicing strict vegetarians; and adapted from "Recipes for a Small Planet" and "Laurel's Kitchen, A handbook for Vegetarian Cookery and Nutrition." Soybean milk values were substituted for milk and milk products. Soybean lecithin values was used in place of egg in mayonnaise, etc.

b. The protein content of total vegetarian diets warrants special comment because of the widespread impression that only protein from animal sources will provide an adequate amino acid composition. This concept is based on the studies of the poor growth rates in rats after the feeding of a single protein, vegetable or animal. Single animal proteins are generally superior to single vegetable proteins, soybean protein being one important exception. However, man or rat eats proteins via various food sources. Previous works conducted in our laboratory and elsewhere (2, 4, 5, 14, 40) have shown that, assuming adequate energy intake, several cereals and legumes can be properly combined so that the amino acid shortfalls of each are complemented. A number of combinations can be satisfactory to meet protein needs in humans: beans with corn or rice, cereals with legumes and green leafy vegetables, peanuts with wheat, wheat and rice with chickpeas and sesame, and so forth. All subjects achieved positive nitrogen balances when 8 g of nitrogen derived from these combination of foods were consumed by each participant. When energy is low, however, dietary protein is preferentially metabolized to provide energy rather than to maintain tissue protein and protein functions.

c. Vitamins. Nutritionists have not reached an agreement as to the parameters which may best reflect the requirement for vitamins. Some consider that the saturation of tissues or of specific enzyme activities and of full normalization of metabolic pathway is desirable. Others place less importance on mild abnormalities of biochemical parameters as long as they are not accompanied by an impairment of health (7).

Inhabitants in CELSS-GD are unlikely to suffer vitamin deficiency since appropriate vitamin supplements are readily available to them.

However, little information is available concerning the level at which excessive intake of vitamins is toxic, even though explicit warnings for both children and adults regarding excessive vitamin A intake, more than 7500 R.E. (25,000 I.U.) on a regular basis are included in NRC-RDA (30).

Also, nutrient toxicity tends to be regarded only in terms of short-term megadose effect. The health effects of subtle chronic toxicities are not known nor defined. Habitual supplementation with vitamin (or mineral) preparations indiscriminately may produce nutrient imbalance or unwarrantable toxicity.

d. Minerals. Within the past few decades remarkable progress has taken place in recognizing the important roles of trace elements in nutrition. Despite their presence in small concentrations in tissues, most known essential trace elements act as catalysts of critical enzyme---mediated biochemical reactions and thus exert profound effects on biological functions necessary for health (27).

As mentioned earlier in this report, the RDA for man have been determined for three of these elements, i.e. iodine, iron and zinc. Ranges of "estimated safe and adequate daily dietary intake" have been summarized for chromium, copper, flouride, manganese, molybdenum and selenium.

The determination for adequate dietary intake of mineral for humans in the total plant food scenario is especially complicated by the interactions occurring among dietary components and by the bioavailability of these minerals in different foods.

Interaction of Dietary Components.

Each individual dietary component consists of a few to a large number of substances; and dietary components rarely act independently of each other. Interactions between two or more dietary components and the

constituents thereof can occur in the diet or at the site of absorption, or during intermediary metabolism or elsewhere. In turn, the human requirement for each nutrient is often influenced by other nutrients or variables in the diet. For example, the requirement for thiamin has usually been related to energy intake, especially when calories were derived primarily from carbohydrates (30).

Some evidence (30) indicates that the requirement for vitamin B₆ is related to the intake of protein. Subjects consuming a high protein diet (150 g/day) required at least 2.16 mg/day of pyridoxine a day to maintain normal tryptophan metabolism, whereas male college students who were fed 54 g of protein required only 0.76 mg of this vitamin (21).

The high protein content (approximately 100 g/day) and unfavorable calcium-to-phosphorus ratio of the American diet leads to increased urinary calcium excretion; these findings support an ample calcium intake above the present RDA for calcium of 800 mg/day (24). In a total plant food setting the protein intake for residents in CELSS-GD would likely be only slightly above the RDA value for protein; present RDA for calcium would be adequate.

Our knowledge regarding interactions in minerals has only begun to unravel. Interactions can be either synergistic or antagonistic. The absorption of iron, for instance, is enhanced by ascorbic acid and animal tissues, but it is adversely affected by calcium and phosphate salts, EDTA, phytates, and tannic acid, tea and antacids. Concurrently, calcium and phosphate interact with each other, and with phytates and antacids, as well as with other nutrients. Furthermore, high intake of cobalt, zinc, cadmium, copper or manganese depress iron absorption due to competition for similar binding sites.

It is apparent that such interactions complicate the assessment of individual candidate plants for use in CELSS. However, they have indicated clearly that the nutritional value of any specific food is relative---not absolute: It depends upon:

1. The content and bioavailability of nutrients in that food.
2. The content and bioavailability of nutrients in other foods in the individual's diet.
3. The nutrient requirements for that individual.

In general, alterations in intake of one class of nutrients may well result in alterations in intake of other nutrients (3). The total plant dietary excludes the use of dairy foods and meat, poultry and seafood. By necessity the consumption of legumes, cereals, and vegetables would be increased. Multiple interactions among nutrients and other factors resulting from such alterations of intake would occur.

Stress.

Stress is defined as "the nonspecific response of the body to any demand" (41). It is anticipated that human habitants in the CELSS Ground Demonstrator will experience tension, anxiety or boredom in varying degrees from physical or mental reasons. Although the nutritional significance of stress is largely unknown, abnormal eating behavior such as loss of appetite, compulsive overeating or interference with fat digestion is often associated with stress.

The Food and Nutrition Board, NAS-NRC (30) suggests that nutrient intake in excess of RDA are unnecessary for usual stress. In contrast, increased urinary nitrogen excretion has been reported as the result of

skeletal muscle protein breakdown to meet the increased demands for energy during times of stress (46). Symptoms of impaired taste acuity due to conditioned Zn deficiency are experienced during stress situations.

Biomedical studies regarding the effects of space flight on various chemical elements were carried out on the nine astronauts who participated in the three Skylab flights of 28, 50 and 84 days in 1973-74. Marked increases in urinary calcium, negative calcium balances and related losses of nitrogen and phosphorus have been reported, despite vigorous exercise regimens in flight. Magnesium, sodium and potassium also indicated negative shifts during flight. These observations have been attributed to the withdrawal of gravitational stress (32, 48).

In a recent report (42), the menu for Space Shuttle Columbia was described as preassembled standard menus providing three meals and supplying 3,000 kcal per person per day. The minimum daily nutritional levels supplied by shuttle menu are essentially the same as the RDA which usually are considered as amounts that exceed the requirements. However, even though the actual energy intake was less, consumption per crewman exceeded recommended levels in varying amounts (Table 3). If we assume that the recommended dietary levels represent the amounts of nutrients desired as well as a needed balance of major nutrients, then some of the ratios among nutrients would have been altered, i.e. protein/calcium, calcium/phosphorus/magnesium, iron/zinc, etc. Granted that these changes are not significant, the compounded effect is not known. Whether this increase in nutrient intake intended to counter the nutritional demand imposed by in flight stress is not clear.

Table 3. Estimated mean daily in-flight nutrient consumption per crewman in Space Shuttle Columbia (42)

	kcal	protein	carbo- hydrate	fat	Ca	P	Na	K	Mg	Fe	Zn
		g						mg			
mean/man day	2,656	106.8	358.6	83.1	1,210	1,706	4,506	3,238	387	27.1	17.6
NASA recom- mended levels	3,000	56			800	800	3,450	2,737	350	18	15
RDA		56			800	800	1,100- 3,300	1,875- 5,625	350	10	15

Bioavailability Studies of Minerals.

Rationale.

The Total mineral content of a food does not necessarily reflect its dietary contribution. Absorption and utilization of individual minerals can be influenced by the solubility and ionization of mineral complexes present in a particular food; competitive antagonism among elements with similar properties and the presence of dietary components which bind it can render it unavailable (17, 25).

Bioavailability of a mineral refers to that proportion of the mineral in food that can be absorbed and retained by an animal and utilized to perform biological functions. Bioavailability varies with food source, preparation and processing treatments and interaction with other dietary components. By far, the largest indirect factors that reduce bioavailability of minerals are those that reduce the intestinal absorptive capacity.

The bioavailability of minerals from plant foods is generally considered to be less than that from animal products. The decreased availability may be explained by the phytate and fiber content of the plant foods. In general, diets rich in fiber and phytate decrease the mineral as well as energy, fat, and nitrogen absorption (27). Decreased absorption may not be a problem when the intake of these nutrients in the diet is sufficiently high but they may be of concern when the intake is marginal or low.

The percent of phytate varies considerably in different varieties and strains of plants. The ranges found in foods were cereals (40-90%), legumes (5 to 72%), fruits (0 to 16%), nuts (12 to 50%), vegetables (0 to 32%), and tubers (5 to 23%).

Phytate and dietary fibers inhibit the intestinal absorption of elements by forming relatively insoluble complexes (20). Elements of particular interest relative to complexation by phytate and other dietary fibers include zinc, iron, copper, manganese, and chromium as well as magnesium and calcium.

a. Calcium. The subject of calcium requirement of human has been under investigation for years. However, efforts to understand the role of dietary calcium have been complicated by the influence of numerous factors, nutritional or otherwise, that in aggregate appear to lack a clear cut relation.

Recent experiments on the influence of dietary factors on calcium metabolism in adult animals and humans have shown that raising protein intake increases calcium excretion while decreasing protein intake has the opposite effect (22). The calciuria effect of high protein diets apparently is due primarily to increased acid production, as sulfate, arising from the oxidation of excess sulfur amino acids (49). Calciuria therefore is more severe when proteins high in sulfur amino acid are consumed. Thus, amounts higher than the present RDA of 800 mg of calcium have been suggested for the prevention of bone disease in man in the U.S. (18, 24) in contrast to 500 mg as the recommended intake for Canada and the United Kingdom or 400-500 mg recommended by FAO/WHO (31).

Calcium is obtained only through absorption from dietary sources. A wide range in absorption rates has been reported in the literature. Several dietary factors are known to influence calcium absorption, i.e. the vitamin D, phosphorus, the calcium-to-phosphorus (Ca:P) ratio, protein,

lactose, magnesium, fat, oxalic acid, and phytic acid (26). It is affected by age, immobilization, hormone system and the nutritional status of the individual.

b. Iron. It has been recognized that the relationship between diet and iron status is not due to the total amount of iron ingested but the amount of iron available for absorption (30). Numerous workers have shown that iron absorption is significantly lower, and dietary iron requirement therefore, higher, in total vegetarian diets than in foods from animal sources, mixed diets and iron salts. It is not only the biological form of iron in the food, but also the composite effect of inhibitors or enhancers in a complete meal that determines nonheme iron availability (27, 28).

Ascorbic acid and meat/fish/poultry (MFP) have shown to enhance absorption of nonheme iron (7, 28). A recent report indicated that the inclusion of 5 fluid ounces of orange juice (75 mg ascorbic acid) in a meal of low iron bioavailability would increase the absorption of iron from 5 percent to approximately 20 percent in the individual with low iron stores (28). In the same report a model to estimate bioavailability of iron has been developed assuming certain relationships, e.g. 1 mg ascorbic acid is equivalent to the enhancing power of 1 g cooked MFP or 1.3 g raw MFP. The strong enhancing effect of ascorbic acid allows the vegetarian to construct diets which promote iron absorption.

Tannic acid in tea and many vegetables, phosvitin in egg yolk, and excessive intake of other trace elements such as zinc, copper and manganese are among the factors that tend to decrease absorption of nonheme iron.

c. Zinc. Zinc is particularly vulnerable in a total vegetarian diet because it is present in lower concentration in plant products and because the fiber and phytin in the whole-grains or legumes inhibit zinc absorption in animals and in human beings (10, 39).

Reinhold, et al (33) suggested that phytate binding is of secondary importance to binding by fiber of zinc and iron. Removal of phytate from bran or whole meals by extraction with HCL or by action of phytase failed to decrease the quantity of zinc bound. Bran surpassed cellulose in ability to bind zinc, perhaps due to the strong affinity of protein in the bran for zinc. Lignin and hemicellulose bound zinc to a similar degree as cellulose (16).

Experimental Procedure.

Three animal experiments were conducted in our laboratory to determine the bioavailability of calcium, iron and zinc respectively. The main objective of these studies is to determine the feasibility of a total plant dietary in CELSS-GD. with particular reference to bioavailability of these minerals.

a. Planning of Diet. Five experimental diets were formulated with the following consideration.

1. Higher plants that have been incorporated in terrestrial diets would be more practical candidates for CELSS-GD.
2. Although the importance of plant diversity has been stressed earlier in this report, realistically the number and kind of plants envisioned in CELSS-GD. could be limited.
3. Diets are adequate in micronutrients and nutrients not being studied.

Therefore, each individual diet consisted of three plant types representing the minimum number for plant diversity. It contained one variety of legume, a grain or starchy vegetable and a green vegetable. Sufficient amounts of starch, fat and vitamins were incorporated to insure an adequate intake of nutrients not being studied and to achieve caloric range (3,600-4,400 kcal/kg) and about 15% of protein (29).

Five combinations are designated as:

Diet 1. Soybeans - potatoes - mustard greens

Diet 2. Peanuts - rice - Chinese pea pods

Diet 3. Split peas - field corn - kale

Diet 4. Navy beans - whole wheat - turnip greens

Diet 5. Chickpeas - oats - broccoli

All diet components were cooked to assimilate human foods and dried to remove their high water content. The plant foods in their original state would have been too bulky to provide sufficient amounts of energy or nutrients. Composition of these diets are presented in Table 4. Individual dietary components and each of the five diets were analyzed for protein and minerals. The protein and mineral contents of the diets are shown in Table 5. Calculated value for other nutrients are summarized in Table 6. The high fat content of peanuts contributed 16.9% of fat to Diet 2. Since we were interested in baseline data, we chose not to use defatted peanuts.

Table 4. Composition of Diets

Component	<u>Diet Number</u>				
	1	2	3	4	5
	g/kg of diet				
Soybean	304				
Potatoes	66				
Mustard greens	75				
Peanuts		326			
Rice		375			
Chinese pea pods		126			
Split peas			303		
Field corn			361		
Kale			91		
Navy beans				306	
Whole wheat				351	
Turnip green				78	
Chickpeas					321.5
Oats					350
Broccoli					105
Corn starch	540	158	200	215	206.5
Crisco	-	-	30	35	2
Vitamin mix	10	10	10	10	10
Choline bitartrate	2	2	2	2	2
DL-Methionine	3	3	3	3	3

Table 5. Protein and Mineral Contents of the Diets

Diet	Prot.	Cu	Fe	Mg	Mn	Zn	Phos.	Ca
mg/kg of diet								
1. Soybeans, potatoes, mustard greens	170.5	4.1	40.6	846	13.5	17.6	2427	2096
2. Peanuts, rice, Chinese pea pods	168.4	3.1	40.0	981	13.8	29.4	2461	1473
3. Split peas, field corn, kale	145.8	3.6	18.6	938	7.2	22.5	2584	2273
4. Navy beans, whole wheat, turnip greens	142.3	2.3	54.8	974	20.5	18.3	2772	2425
5. Chickpeas, oats, broccoli	187.1	6.2	49.4	1273	30.3	29.3	3828	1675
5% salt mixture (37)		19.0	49	490	19.5	4.0	3740	5920
NRC (29)		5.0	35	400	50.0	12.0	4000	5000

Table 6. Nutrients in 1.0 kg of Experimental Diets.

Diet	Energy	Fat	Total	Fiber	Ash	K	Vitamin	Thiamin	Riboflavin	Niacin	Ascorbic acid
			CHO				A				
			g			mg	I.U.		mg		
1. Soybeans, potatoes, mustard greens	3870	66.4	692	29.5	26.2	9254	64280	10.6	8.4	47.7	410
2. Peanuts, rice, Chinese pea pods	4405	169.4	618	21.3	21.8	4584	10800	14.3	7.7	102.5	210
3. Split peas, field corn kale	3783	52.2	712	20.4	22.2	6537	88135	11.1	9.2	56.2	640
4. Navy beans, whole wheat, turnip greens	3701	51.3	695	33.4	27.9	7361	73000	10.9	8.3	58.5	340
5. Chickpeas, oat, broccoli	3363	46.7	610	33.8	24.3	6462	23175	9.9	8.3	46.5	780

b. Experimental Animals. Male rats of the Sprague-Dawley strain were used in all of the studies. Animals were approximately 8 weeks old and weighed between 185 and 224 grams upon arrival. One hundred and twenty rats were randomly assigned to the five total vegetarian diets; then subdivided for bioavailability studies of Ca, Fe, and Zn.

All rats were housed separately in plexiglass cages with elevated rod bottoms to facilitate the collection of feces and minimize coprophagy.

The animals were stabilized with the experimental diet for 4 days. After an 18-hour fasting, rats were given the extrinsically labeled isotope: ^{47}Ca , ^{59}Fe or ^{65}Zn with 3 g of food. The rats then were given non-isotope experimental diet and counted daily in a whole body counter for the following 12 days. Absorption rates were determined by measuring the respective radio isotopes retained in the animal each day.

Results.

The rates of absorption of Ca, Fe and Zn in the five experimental diets are presented in Fig. 1, 2, 3 respectively. Diet 2, the peanuts - rice - Chinese pea pods combination was better absorption for all the three minerals studied. Animal studies showed that moderate amounts of fat favored absorption of minerals (26). The effect of 16.9% of fat intrinsically present in Diet 2 seems to support this finding.

The amount of calcium in the experimental diets varied from 1,473 to 2,425 mg per kg of diet. These values are markedly lower than the proportion of 5,920 mg of calcium per kg of diet used in basal diets as optimal intake (37) or the 5,000 mg/kg diet recommended by NRC (29). The percent of calcium absorption is ranked against the calcium contents in the respective diets in Table 7. An inverse relationship is clearly shown.

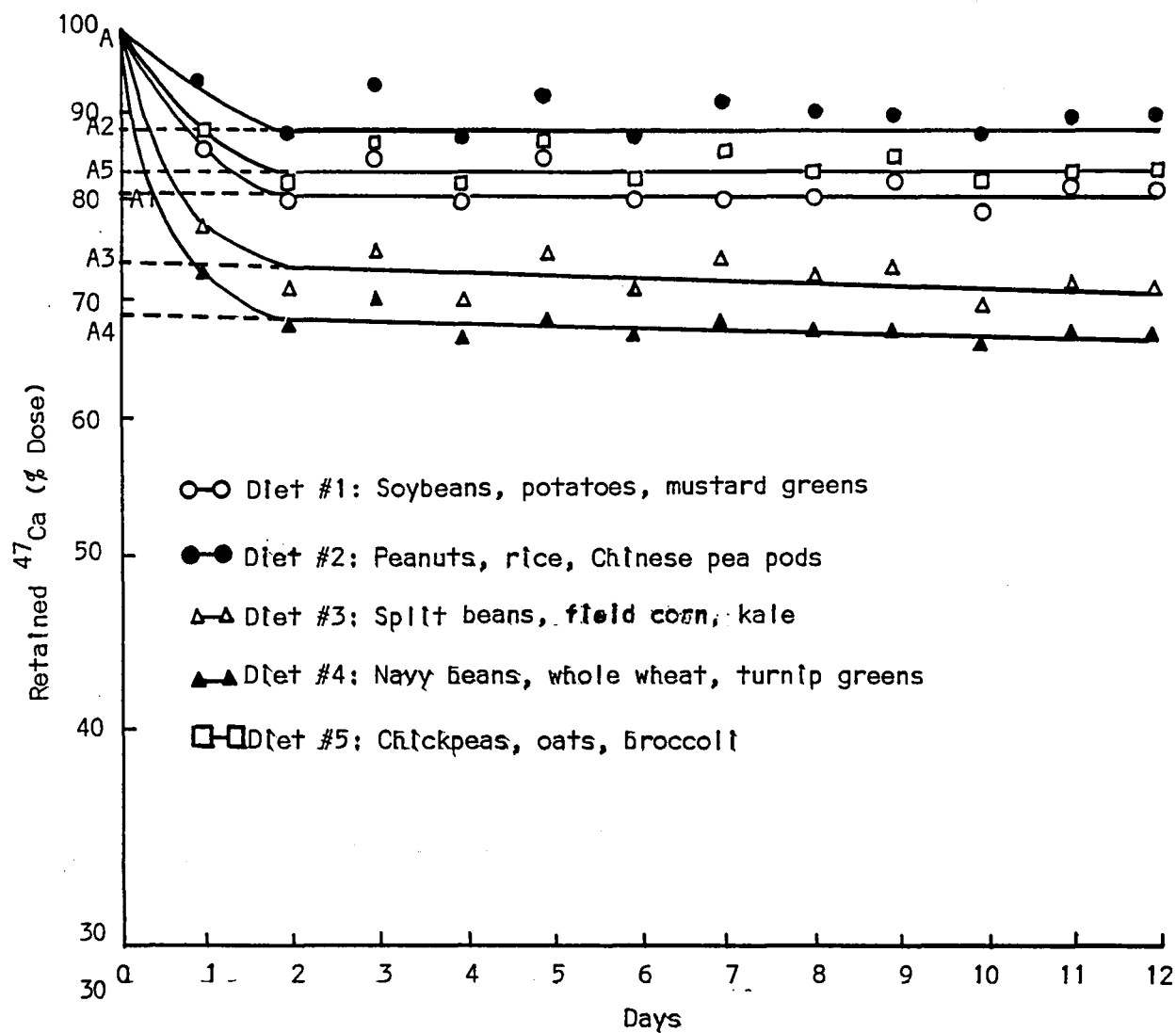


Fig. 1 Whole body radioactivity of ^{47}Ca in five total vegetarian diets. The account is arbitrarily assigned 100% at time 0 (A). The back extrapolated to time zero is shown as the dotted line to (A1).

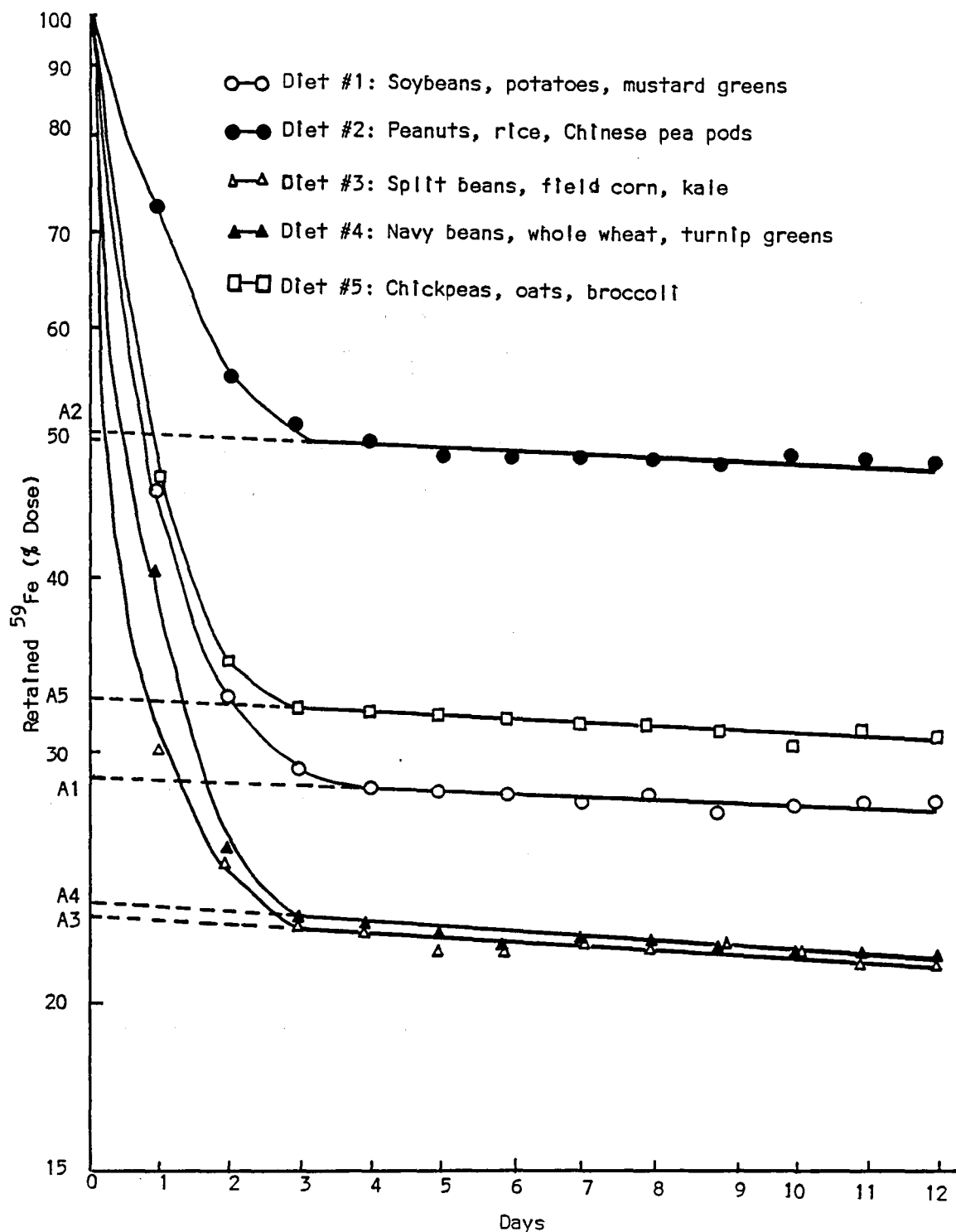


Fig. 2 Whole body radioactivity of ^{59}Fe in five total vegetarian diets. The account is arbitrarily assigned 100% at time 0 (A). The back extrapolated to time zero is shown as the dotted line to (A1).

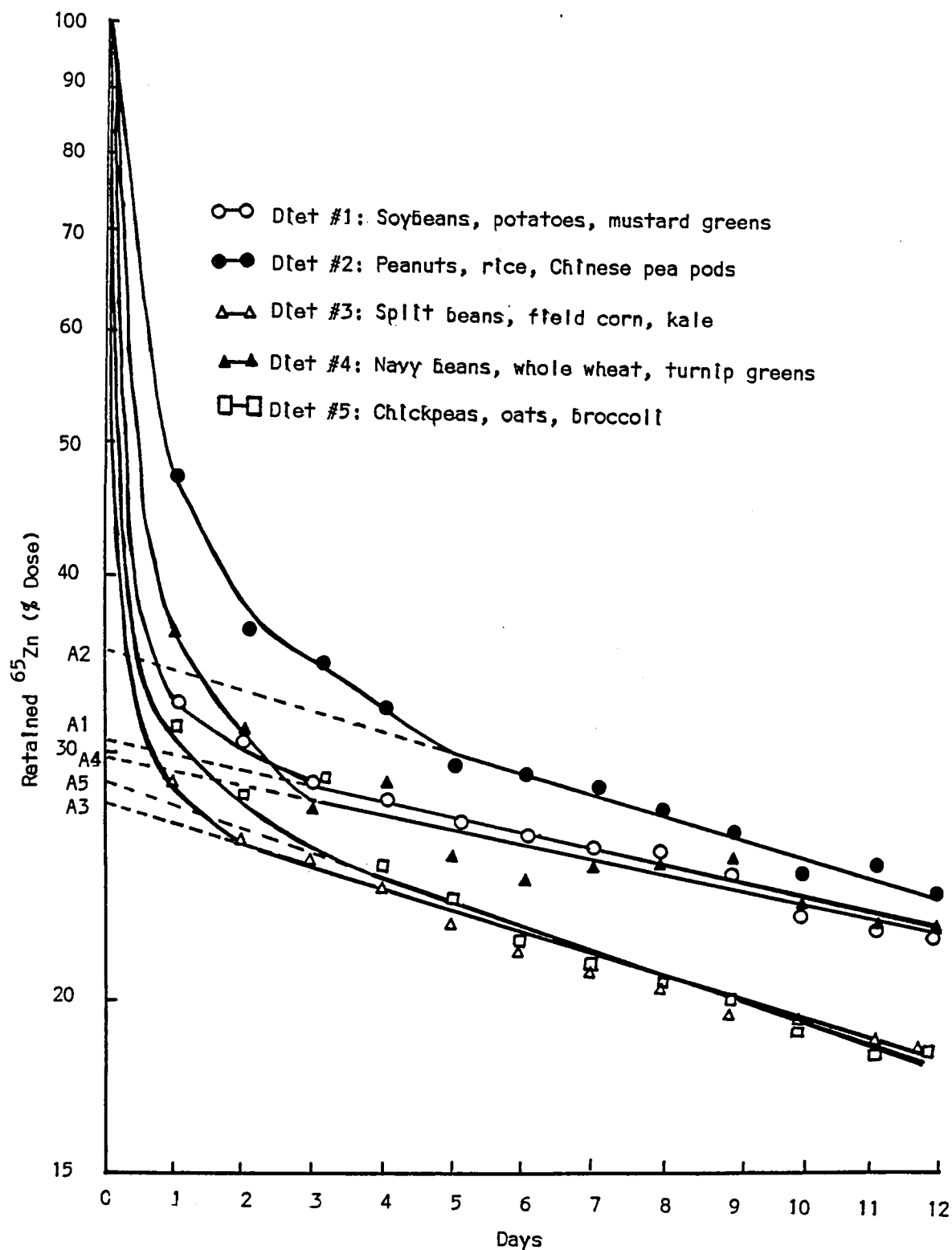


Fig. 3 Whole body radioactivity of ^{65}Zn in five total vegetarian diets. The account is arbitrarily assigned 100% at time 0 (A). The back extrapolated to time zero is shown as the dotted line to (A).

Table 7. Calcium absorption in Sprague-Dawley rat (8 weeks old)

Dietary component	Calcium absorption %	Calcium content mg/kg
Peanuts, rice, Chinese pea pods	89	1473
Chickpeas, oats, broccoli	83	1675
Soybeans, potatoes, mustard greens	80	2096
Split peas, field corn, kale	71	2273
Navy beans, whole wheat, turnip greens	65	2425

These data indicated that when calcium intake is marginal, homeostatic regulation operates for intestinal calcium uptake. This phenomena acts in concert with the "mucosal block" theory. It postulates that unneeded mineral, although taken up by the enteric cells, will be withheld from passing into the body, while only the amounts needed to maintain normal balance or to reverse depletion will be permitted to enter the blood stream.

The effect of diets on Ca and Fe absorption is similar within their respective range. The velocity of absorption was highest in the peanut, rice and Chinese peapods combination followed by the chick peas-oats-broccoli, then the soybean-potatoes-mustard green combination. Calcium absorption in the split peas-field corn-kale combination was better than that of the navy beans-whole wheat-turnip green combination. The Fe absorption of the latter two diets was virtually the same. Because foods were dehydrated the zinc concentration of these diets were high. On the other hand, since part of the energy of the diet was supplied by corn starch, the amounts of fiber in the diets were relatively modest.

The effect of diets on Zn absorption was not as distinct as that on either calcium or iron. Since zinc has a number of accessible excretion routes the physiological control to the overload of zinc in these diets was not as restricted at the level of intestinal absorption.

The moderate amounts of existing fiber in the diets did not appear to affect mineral absorption significantly. This finding is in agreement with the report of Story (44) who has suggested that the main interactions between dietary fiber and intestinal contents are water holding, absorption

of solutes and modification of intestinal flora. Few changes in mineral balance occur when moderate amounts of dietary fiber are added to a mixed diet.

The enzyme phytase, found in many cereals, destroys phytic acid. Legumes and cereals used in the experimental diets were soaked in water and then cooked. Whether some of the phytic acid was destroyed during the soaking and cooking process or whether the amounts of calcium, iron and zinc in the diets are sufficient to compensate for the moderate proportion of phytic acid in the diets is not clear. Absorption rates did not appear to be affected significantly by the phytates in these diets as compared to other studies.

Experiment IV

Soybean protein has become a replacement for animal protein as a meat and/or milk substitute in practicing vegetarian diets. A number of studies have indicated that soy bean protein in the diet will suppress the utilization of calcium, phosphorus, magnesium, zinc, copper and manganese. In the three experiments described earlier the effect of soybean-potatoes-mustard green diet was not as favorable on mineral absorption as some of the other combinations.

Both calcium and magnesium are divalent ions and both markedly influence parathyroid hormone (PTH) stimulation yet they are antagonistic in many physiological processes.

In a total vegetarian diet intake of protein is expected to be lower while intake of magnesium would be higher. Since protein and magnesium are known to influence calcium utilization this experiment in essence extends the Ca absorption study. Experiment IV was conducted to examine the effect of levels of protein (soy protein) or magnesium and their interaction on calcium metabolism (15).

A 3 x 3 factorial design was used in planning the experiment. A combination of three levels of protein and three levels of magnesium was used to compose the 9 experimental diets. The combinations represented low, medium and high levels of protein designated as Pl, Pm and Ph respectively. Similarly, low, medium and high levels of magnesium were designated as Ml, Mm, and Mh respectively. The low protein level of 5.5-5.9%, Pl, was chosen because in a total-vegetarian diet protein level would be low. The protein level of 10.6-10.7%, Pm, represents the amount recommended by the National Research Council, whereas the protein level of 21.4-22%, Ph, represents an excessive protein consumption.

The magnesium level of 0.053%-0.064%, Ml, was derived from the levels which are naturally present in the food ingredients used, i.e. kale, turnip greens, rice, and small amounts of soy beans, etc. MgSO_4 was added to Mm and Mh diets to raise the Mg levels of these diets to 0.084-0.095% and 0.14-0.15% respectively.

The experimental diets were formulated by replacing portions of corn starch with soy assay protein. Thus, the amount of magnesium in Ml diet is low since the purified soy protein or the corn starch contains negligible amounts of trace elements including magnesium. The proportion of magnesium in Mm diets may represent those in mixed diets; whereas in a total vegetarian diet the proportion of Mg would be more closely represented by the Mh diet (Table 8).

All diets contained the same amounts of minerals (except magnesium), vitamin-dextrin mixture and fat. The protein and mineral contents of the diets were determined and are summarized in Table 9.

Table 8. Composition of Diets in Experiment IV

	Type of Diet								
	P1M1	P1Mm	P1Mh	PmM1	PmMm	PmMh	PhM1	PhMm	PhMh
	Ingredient (gm/kg of diet)								
Kale ¹	70	70	70	70	70	70	70	70	70
Turnip greens ¹	55	55	55	55	55	55	55	55	55
Soybean ²	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Rice ³	200	200	200	200	200	200	200	200	200
Corn starch ⁴	603	601	598	543	541	538	423	421	418
Fat ⁵	50	50	50	50	50	50	50	50	50
Vitamin mix ⁶	10	10	10	10	10	10	10	10	10
DL methionine ⁷	3	3	3	3	3	3	3	3	3
Choline bitartrate ⁸	2	2	2	2	2	2	2	2	2
Soyassay protein ⁹	-	-	-	60	60	60	180	180	180
MgSO ₄ ¹⁰	-	1.74	4.64	-	1.74	4.64	-	1.74	4.64

1 Birds Eye Brand, General Food Corporation.

2 Amysoy 70.

3 Uncle Ben's Converted Rice, long grain enriched parboiled.

4 Argo, Best Foods CPC International Inc.

5 Crisco, Dehydrogenated vegetable oil.

6 Vitamin premix supplies amount per kg diet: thiamin HCl, 0.6 g/kg; riboflavin, 0.6 g/kg; pyridoxine HCl, 0.7 g/kg; niacin, 3.0 g/kg; calcium pantothenate, 1.6 g/kg; folic acid, 0.2 g/kg; biotin, 0.02 g/kg; vitamin B₁₂, 1.0 g/kg; dry vitamin A palmitate (500,000 μ /g), 0.8 g/kg; vitamin D₃ trituration (400,000 μ /g, 0.25 g/kg; dry vitamin E acetate (500 μ /g), 10 g/kg; menadione, 0.005 g/kg; sucrose, 981.225 g/kg.

7 USB, Cleveland, Ohio

8 ICN Pharmaceuticals, Inc. Cleveland, Ohio

9 Soyassay protein, Alcohol water extracted. Teklad, Division of Harland Indiana Inc.

10 AR. Mallinckrodt, Inc.

Table 9. Protein and Mineral Content of Experimental Diets in Experiment IV

Diet	Protein (%)	Ca (%)	Mg (%)	Cu (%)	Fe (%)	Mn (%)	Zn (%)	P (%)
Plml	5.9	0.29	0.053	N.D. ¹	0.0047	0.0005	0.0012	0.10
PlMm	5.8	0.27	0.084	N.D.	0.0033	0.0001	0.0014	0.10
PlMh	5.5	0.29	0.140	N.D.	0.0040	0.0001	0.0022	0.10
PmMl	10.7	0.29	0.055	N.D.	0.0054	0.0005	0.0013	0.13
PmMm	10.6	0.30	0.090	N.D.	0.0061	0.0005	0.0037	0.13
PmMh	10.6	0.30	0.145	N.D.	0.0054	N.D.	0.0013	0.13
PhMl	21.4	0.34	0.064	N.D.	0.0111	0.0005	0.0022	0.20
PhMm	21.9	0.34	0.095	N.D.	0.0097	0.0001	0.0019	0.21
PhMh	22.0	0.35	0.150	N.D.	0.0231	0.0005	0.0021	0.21

¹ N.D. not detected.

Seventy-two Sprague-Dawley strain male weanling rats were randomly assigned to the diet groups. Absorption of calcium were determined by measuring ^{47}Ca retained in the body. Calcium and magnesium concentration in femure bones were measured by atomic absorption spectrophotometry after rates were fed test diets for 35 days.

Results of Experiment IV indicated the following:

1. Levels of protein per se or magnesium did not have a dis-
concerning effect on calcium absorption.
2. High amounts of protein in the diets stimulated food con-
sumption and weight gain of the animals, while the increase
of magnesium in the diet suppressed food intake and body
weight gain.
3. Calcium intake linearly correlates with the total calcium
content in femure bones.
4. Animals in the Pl, Mh group which represents vegetarian
diet of low protein, high magnesium intake had the lowest
calcium intake, and the lowest femur bone weight; and the
lowest total calcium content in femur bones among the nine
diet groups studied.

Summary.

The preceding literature review and research may be summarized as follows:

- . If we assume that the inhabitants of CELSS-GD will live under mildly stressful conditions the present known RDA are adequate to meet the nutrient requirements.
- . A total plant dietary can be satisfactory, provided a diversity of plants will be available for use in CELSS-GD. Some supplements for micronutrients may be necessary. However, indiscriminate use of vitamins and minerals preparation is to be discouraged.
- . The minimum number of three plant species per candidate group is not adequate to provide a balance of nutrients. Imbalance of minerals were shown in the animal experiments conducted.
- . More research effort is needed to target on -
 - a. the inter-relationship of nutrients.
 - b. long duration human feeding experiments under controlled conditions.

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APPENDIX A

A 14-Day Total Vegetarian Cycle Menu

DAY 1

Breakfast

2	Buckwheat pancakes
1 C	Cinnamon-flavored applesauce
1 slice	white bread toasted
1 C	Red clover blossom tea

Lunch

1	Sandwich of soy pate
2	Cucumber slices
1	Apple
1 C	Soy milk
1 slice	whole-grain bread

Dinner

1/2 C	steamed greens
1	Savory squash pie
1 C	Soy milk
1	Green Beans Hellenika
1	Tomato pepper salad

DAY 2

Breakfast

1	English Muffin
2	wheat toasted
1	Dilled cucumber and yogurt salad
1 C	Mint Tea

Lunch

1 C	Tomato soup
4	Sally's savory Crackers
1/4 C	Celery sticks
1/4 C	split pea
1/4 C	tofu
1 C	Soy milk

Dinner

3	Tennessee Corn Pone
1	Broccoli spears with yeast margarine
1 T	French Dressing
1/2 C	Tossed Salad (spinach)
1	Diana's Apple Crisp
1 C	Tea

DAY 3

Breakfast

4 Sarah's Sourdough Pancakes
 1 T Hot orange sauce
 1 C Ganhis Coffee
 1 C Fresh strawberry yogurt

Lunch

3 T Jessica's Mock Rarebit over
 2 slices whole-grain taost
 1 Sunchoke salad
 1 apple

Dinner

1 New England baked beans
 1/2 C Asparagus spears
 2 Boston Brown Bread
 1 Summer Salad
 1 C Soy milk
 1 slice strawberry-Rhubarb pie

DAY 4

Breakfast

1/2 C sliced raw peaches
 1/2 C oatmeal with
 1/2 C Soybean milk
 1 T honey
 1 slice whole-grain bread
 2 T peanut butter
 1 C Tea

Lunch

1 C Manybean soup
 4 Corn bread
 1 Chef's salad
 1 C Soybean milk

Dinner

1 Creamy sesame beans and celery
 2 Potato poppens
 1 Coleslaw
 20 pieces French-fried

DAY 5

Breakfast

1/2 C	Oatmeal with
1/2 C	Soy milk
1 T	Maple Syrup
2	Apple Bran Muffins
1 C	Rose hips tea

Lunch

2	Soy Burgers
2 slices	white bread
1/4 C	Lettuce
1 C	Soy milk

Dinner

1	Shades of Green Salad
3	Crispy Seed Wafers
1/2 C	orange juice (fresh)

DAY 6

Breakfast

4	pancakes
---	----------

Lunch

1	Sesame Tomatoes on Rice
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Dinner

2	Nut and seed patties
1	Baked Potato
2 slices	wheat flour toast
1	Banana
1 C	Coffee
1 T	Sugar

Breakfast

1 Victory breakfast

Lunch

1 Brown rice buffet
 1 C Coffee
 1 T sugar

Dinner

1/2 C Marinated white beans
 2 slices Garlic Bread
 1 Tossed green salad with zucchini
 1 Walnut cake

DAY 8

Breakfast

1 orange, medium
 1 C bulgur with
 1 T brewer's yeast
 1 slice Toasted wheat bread with
 1 T honey

Morning Snack

1/4 C shelled almonds

Lunch

2 C Split pea soup
 2 T peanut butter
 2 slices whole wheat bread
 1 T honey
 1 Fruit-Sunflower Seed Salad

Afternoon Snack

1 peach, medium

Dinner

1 C Soybean
 1 C Brown rice cooked with
 2 T Corn oil
 2 T Chestnut
 2 T Sesame Seeds
 1 C Collard
 1 Pear

Evening Snack

1/4 C Raisins

DAY 9

Breakfast

1 C	Soy milk
2 slices	White flour bread
2 T	margarine
1	banana
1 t	white sugar
1 C	Tea

Lunch

1 C	Cooked long grain brown rice
1 1/2 C	Soy bean and
1/2 C	winter squash
1/4 C	peanuts, roasted
1/6 wedge	watermelon
2 t	white sugar
2 T	corn oil
1 C	Tea

Dinner

1 C	Cooked long grain brown rice
1 C	bean sprouts
1 T	soy sauce
1/4 C	celery
1/4 C	carrots
1 C	soy milk
1	orange, medium
2	walnut cake
1 C	Tea

DAY 10

Breakfast

1/2 C	tomato juice
2	wheat bread
2 T	maple syrup
1 C	soybean milk

Lunch

1	banana
1/2 C	tomato soup
1	baked potato
1/2 C	apple
1/2 C	celery salad
2 T	french dressing
1/2 C	peas, cooked
2 t	white sugar
1 C	Tea

Dinner

2 slices	wheat bread
2 T	margarine
1/2 C	baked beans (lima)
1/2 C	carrots
1/2 C	spanish rice
1/2 C	onion
2 T	Thousand island dressing
1	peach, medium
2	Italian bread
1	orange
1 t	sugar
1 C	Tea

DAY 11

Breakfast

3/4 C	oatmeal
1/2 C	Raisins
1/2 C	orange juice
1 t	brown sugar
1 C	coffee

Lunch

1	Peanut Butter and Jelly Sandwich
1	apple
1 C	soy milk
1/2 C	cole slaw
2 C	whole wheat roll
1 t	brown sugar
1 C	coffee

Dinner

1	baked potato
1/2 C	spanish rice
1/2 C	yellow brans
1 C	strawberry
1/2 C	applesauce
1 t	brown sugar
1 C	coffee

DAY 12

Breakfast

1/2 C	orange juice
1/2 C	cream of wheat cereal
1 slice	toasted bread
1 t	margarine
1 T	grape jelly
2 t	sugar
1 C	Coffee

Lunch

2 T	peanut butter
1	baked potato
1/2 C	greenbeans
1/2 slice	tomato on lettuce
1 slice	Vienna bread
2 T	margarine
1/2 C	peach
1	puffed rice bar
1 t	sugar
1 C	Tea

Dinner

3 oz	soy meat analogue
1/2 C	French Fried
1/2 C	cooked carrots
2 large	lettuce
1 T	French dressing
1 slice	bread, white
1 t	margarine
2 t	sugar
1 C	coffee

DAY 13

Breakfast

1 C fresh grapefruit juice
 1 C Hot oatmeal with
 1/2 C soy milk
 1 slice wheat grain bread
 1 T peanut butter

Lunch

1 vegetable soup
 2 slices wheat grain bread
 2 T margarine
 1 Fruit salad with sunflower seeds

Dinner

1 Rice patties
 1/2 C baked winter squash
 1/2 C green bean with
 1/8 C raisins
 1 slice strawberry-Rhubarb pie

DAY 14

Breakfast

1/2 C orange juice
 2 whole wheat grain pancakes with
 3 T maple syrup
 1 apple

Lunch

1 sprout salad
 1 C soybean milk
 1 banana

Dinner

1/2 C Deep-fried tofu
 1 C steamed rice (brown long grain)
 1 mixed stir-fried vegetables
 1 C soy milk

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1. Report No. NASA CR-166357		2. Government Accession No.		3. Recipient's Catalog No.	
4. Title and Subtitle Plant Diversity to Support Humans in a CELSS Ground-Based Demonstrator				5. Report Date June 1982	
				6. Performing Organization Code	
7. Author(s) Howe, J. M. and J. E. Hoff				8. Performing Organization Report No.	
9. Performing Organization Name and Address Departments of Food and Nutrition and Horticulture Purdue University West Lafayette, IN 47907				10. Work Unit No. T5992	
				11. Contract or Grant No. NSG-2401	
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration Washington, D.C. 24056				13. Type of Report and Period Covered Contractor Report	
				14. Sponsoring Agency Code 199-60-62	
15. Supplementary Notes Leonard P. Zill, Technical Monitor, Mail Stop 239-10, Ames Research Center, Moffett Field, CA 94035 (415) 965-5573, FTS 448-5573. The 11th in a series of CELSS reports.					
16. Abstract This report considers factors that influence the human nutritional requirements envisioned in a controlled ecological life support system (CELSS) ground-based demonstrator. Also discussed are experiments concerning the bioavailability of Ca, Fe, and Zn, and the interrelationship of protein and Mg in Ca retention.					
17. Key Words (Suggested by Author(s)) CELSS Life Support Systems Human Nutrition Mineral Bioavailability Food Production				18. Distribution Statement Unclassified - Unlimited STAR Category 54	
19. Security Classif. (of this report) Unclassified		20. Security Classif. (of this page) Unclassified		21. No. of Pages 51	
22. Price*					

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